Survey of lettuce for potential perchlorate accumulation

A Report Submitted to the Arizona Iceberg Lettuce Research Council

Submitted by

Charles A. Sanchez
Professor of Soil, Water, and Environmental Sciences
Yuma Agricultural Center

Introduction

The Colorado River is contaminated with low levels of perchlorate. This contamination is from rocket engine or fuel industries via the Las Vegas wash. Furthermore, there is some concern that food crops irrigated with this water may bioaccumulate perchlorate. Perchlorate is linked to thyroid dysfunction and considered a health threat to humans (Clark, 2000).

The production of fresh market vegetables (lettuce, broccoli, cauliflower, etc) in the lower Colorado regions of Arizona and California is a multi-million dollar industry. Essentially 100% of this industry relies on Colorado River water for irrigation.

Recent greenhouse studies have shown a potential for lettuce to bioaccumulate perchlorate (Hutchinson et al., 2000). Based on this greenhouse study it has been proposed that health risk considerations assign a 40% relative source contribution to lettuce. However, these studies were not performed under realistic vegetable growing conditions in the low desert. First, these evaluations used perchlorate concentrations several times that present in Colorado River irrigation water. In addition, these studies did not consider competing effects of other anions. These anions include Cl^-, SO_4^{-2}, HCO_3^-, F- and NO_3^-, which are typically present in Colorado River water at concentrations of 120 mg/L, 300 mg/L, 180 mg/L, 0.4 mg/L, and 0.2 mg/L, respectively. Several studies have shown that plant accumulation of perchlorate, and its analog pertechnetate, is affected by the presence of other anions (Cataldo et al., 1978; 1983; 1986; Echevarria et al., 1997; 1998; Kriger et al., 2000; Nzengung et al., 1999). Furthermore, these studies were performed in solution culture and ignored the effect of the soil, where concentrations of many ions in the soil solution are sometimes altered from irrigation water through physical and chemical interactions with the soil matrix and agricultural management. For example, lettuce is typically and adequately fertilized with N and it is suspected that nitrate is among the anions that inhibit perchlorate uptake.

The objective of this project is to conduct a preliminary survey of the potential for desert lettuce to bioaccumulate perchlorate.

Materials and Methods

Lettuce fields at maturity were identified and permission was obtained for sampling. Fields in the south Yuma Valley were sampled February 6, fields in the Gila Valley (North and South) were sampled February 18, and fields in the Wellton Mohawk Irrigation and Drainage District were sampled February 25. Ten field sites were sampled in the south Yuma valley, and seven field sites in each the Gila and Wellton-Mohawk regions. After recording the location, we took seven whole plants at random from each 40-acre field and transported them to our laboratory. Three plants were processed whole. Four plants were partitioned into wrapper (and frame) leaves and edible head. The weights of each portion were recorded. These samples were then diced, mixed thoroughly, and a sub-sample was placed in the freezer. At a latter time the frozen sample was freeze dried. Weights before and after freeze-drying were recorded. Lettuce typically took 48 hours for complete freeze-drying. The samples were ground and stored in vials for extraction.

We used an extraction procedure described previously (Ellington and Evans, 2000) with minor

modifications. Briefly, 600 mg of freeze-dried product was weighed out into centrifuge tubes and 15 mL of DI water were added. The tubes were boiled for 30 minutes and the contents were placed in a refrigerator overnight with occasional gentle shaking. The tubes were then centrifuged for 30 minutes and the supernatants filtered through Kim wipes and filtered through 0.2 um Gel man ion membrane syringe filters. This solution was stored in vials labeled "extract one". Two mL of the above extract (extract one) was reacted with 1000 mg DD-alumina overnight in vials. Vials were gently agitated two or three times over 24-hour period. Eighteen mL if DI water were then added to this mixture. After stirring and settling, this solution was filtered through another 0.2 um Gel man ion membrane syringe filter and the resulting solution was labeled "extract 2". This sample was stored in the freezer until analysis by ion chromatography (IC). Before loading on IC this extract was allowed to reach room temperature and was filtered through a Dionex On Guard RP syringe filter. The On Guard filters had been pre-cleaned first with methanol then with DI water. Furthermore, the first 0.75 mL of sample (extract 2) pushed through the filter is discarded and the remaining aliquots used for IC analysis.

As a standard practice we would run10% duplicate extractions and 10% spiked additions. However, for these lettuce samples we actually exceeded this number of extractions because we were developing our methodology and we processed samples on more than one IC. Duplicate aliquots of a given extraction were always analyzed. Additional aliquots were analyzed if we judged variability on the first two aliquots excessive.

Because our laboratory did not have an IC when these studies were initiated, samples were initially run on a Dionex 320 located at Sandia National Laboratories (SNL). The Dionex 320 at SNL used the 4 mm AG11/AS11 guard and separation column pair, 95 mM KOH isocratic eluent generation and 100 mM sulfuric acid suppression using the AMMS III.

By August our Soil and Water Research Laboratory at the University of Arizona's Yuma Agricultural Center purchased a new Dionex 2500. The Dionex 2500 contains an IP 25 isocratic pump, an EG50 eluent generator, a CD conductivity detector, the 2 mm AG16/AS16 guard and separation column pair, and an AMMS III suppressor. The columns, suppressor, and detector are housed in a LC 30 chromatography oven. We used a 50 mM KOH eluent and 50 mM sulfuric acid suppression. A 1000 uL injection loop was used and elution time ranged from 9.5 to 10.9 minutes.

We calibrated with standards ranging from 0.5 to 100 ug/L. The coefficient of determination was greater than 0.99. Ideally, one should calibrate in matrix, but this is difficult to do for environmental and biological specimens because matrices are not constant. Therefore, we guarded against matrix errors by spiked additions. Approximately 10% of the lettuce samples were extracted with a 100 ug/L perchlorate standard to yield 10 ug/L perchlorate after dilution.

All lettuce samples were run on both IC units. Generally, the data from both instruments was in agreement. However, because we found the sensitivity better on the Dionex 2500, nearly all data reported in this summary were analyzed on this instrument.

In addition to plant material we obtained sub-samples of composite Colorado River water samples collected by the U.S. Bureau of Reclamation (USBOR). The USBOR and the

International Boundary Commission collect these samples twice monthly for water quality assessments. The analysis performed on these samples by the USBOR includes salinity and all major cations and anions but they do not analyze for perchlorate. These were analyzed for perchlorate in our laboratory following EPA Method 314. A total of 14 water samples were collected between March and August of 2003.

Results and Discussion

The method detection limit (MDL) was determined using the procedure outlined in EPA method 314.0 (USEAP, 1999) using seven replicates of a standard in reagent water. The calculated MDL was 0.2 ug/L using a 0.5 ug/L standard. The minimum reporting level (MRL) is defined as the concentration that can be reported as a quantitated value. It is recommended that the MRL be established at an analyte concentration greater than 3 times the MDL or at a concentration that would yield a signal to noise ratio grater than 5. Furthermore, the MRL must never be established at a concentration lower than the lowest standard. For example, an MDL of 0.53 ug/L and an MRL of 4 ug/L is reported for reagent water in EPA Method 314.

Using the aforementioned criteria we have tentatively set our MRL at 2 ug/L. With our typical extraction and dilution ratio this corresponded to a dry weight concentration for perchlorate of 500 ng/g. The concentration on a wet weight basis depends on the moisture composition of the plant. For example, if the lettuce is 5% dry matter this would correspond to 25 ng/g on a fresh weight basis. However, for plant material at 10% dry matter this would correspond to 50 ng/g on a fresh weight basis. The average dry matter content for all the lettuce material sampled in this survey was 6%; therefore, we tentatively set 30 ng/g as our MRL on a fresh weight basis. This value may ultimately be adjusted as we collect more information on instrument performance and conduct a more rigorous statistical evaluation of this performance.

Overall, for lettuce we found reasonable agreement among duplicate extractions and duplicate aliquots. The relative standard deviations among aliquots averaged12% and the relative standard deviation among duplicate extractions averaged18%. Our recovery of spikes in the lettuce matrix fell within limits described in EPA Method 314.0.

The data for whole above ground lettuce plants are shown in Table 1. Overall, the perchlorate content of the whole above ground plant was below the MRL for most of the samples collected. Of the 24 sites sampled, only seven whole-above ground lettuce plant samples exceeded the MRL. The values less than 30 ng/g reported in Table 1 were from samples where the extract gave values of 2 ug/L or above on the IC but % dry matter was less than 6.

The data for perchlorate content in the partitioned lettuce plant is shown in Table 2. The frame leaves are typically left in the field after harvesting and the grocer and/or consumer trim the wrapper leaves. The edible core represents the portion typically consumed. Interestingly a high percentage of the samples collected showed detectable perchlorate in the combined frame and wrapper leaves. In fact, most had levels above the MRL. Conversely, no head had perchlorate levels that exceeded the MRL and several were below our detection limit altogether. These data clearly indicate that while perchlorate accumulates in iceberg lettuce it is largely in the outer leaves. It is likely that perchlorate moves into plants in the transpiration stream and it

accumulates as water transpires through the leaves (Ellington et al., 2001; Sunberg et al., 2003). For iceberg head lettuce, transpiration largely occurs in the outer leaves.

Urbansky and Brown (2003) reported that perchlorate in soils was largely depositional rather than sorptive. In other words, for the situation in the lower Colorado Region, perchlorate is transported into and through soils with irrigation water with little or no physical or chemical sorption by the soil. It is possible for perchlorate to temporarily accumulate in the crop-rooting zone when evapotranspiration exceeds leaching. Nevertheless, because lettuce is salt sensitive growers typically apply irrigation water to achieve leaching fractions to preclude detrimental salt accumulation. Therefore, over a growing period the perchlorate concentration of the irrigation water is a reasonable estimate of plant availability. The concentration of perchlorate in the composite river samples ranged from 3 to 6 ug/L. Although none of these samples were collected during the growing season of the lettuce plants sampled, they generally agree with previous samples collected by others and should represent a reasonable estimate of perchlorate availability in irrigation water.

It is likely irrigation water is the major source of perchlorate in lettuce. However, because perchlorate can sometimes occur in some fertilizers and amendments (Urbansky et al., 2001; Orris et al., 2003) other sources cannot completely be ruled out. Furthermore, the variation across locations suggests that management factors may influence perchlorate accumulation in lettuce. Additional work is needed to study factors affecting perchlorate uptake and distribution in lettuce. Additional work is also needed to evaluate perchlorate accumulation in other lettuce types and leafy vegetables. Finally, work is needed to assess health risks associated with the consumption of desert lettuce.

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Table 1. Perchlorate content in whole-above ground plants samples in the lower Colorado River region of Arizona.

Sample	**Sample Location**	**Perchlorate concentration (ng/g) fresh weight**
		Whole above-ground plant
1	Yuma Valley	37
2	Yuma Valley	32
3	Yuma Valley	<MRL
4	Yuma Valley	<MRL
5	Yuma Valley	27
6	Yuma Valley	<MRL
7	Yuma Valley	<MRL
8	Yuma Valley	<MRL
9	Yuma Valley	<MRL
10	Yuma Valley	33
11	Gila Valley	<MRL
12	Gila Valley	29
13	Gila Valley	27
14	Gila Valley	<MRL
15	Gila Valley	<MRL
16	Gila Valley	<MRL
17	Gila Valley	<MRL
18	Wellton-Mohawk	30
19	Wellton-Mohawk	<MRL
20	Wellton-Mohawk	<MRL
21	Wellton-Mohawk	<MRL
22	Wellton-Mohawk	<MRL
23	Wellton-Mohawk	<MRL
24	Wellton-Mohawk	<MRL

<MRL represents seemingly detectable peak but below level that can be quantitated.

The values less than 30 ng/g reported above were from samples extracts that gave values of 2 ug/L or above on the IC but dry matter was less than 6%.

Table 2. Perchlorate content of the combined frame and wrapper leaves and edible head in lower Colorado River Region of Arizona.

Sample	**Sample Location**	**Perchlorate concentration (ng/g) fresh weight**	
		Frame-Wrapper-Leaves	**Head**
1	Yuma Valley	94	<MRL
2	Yuma Valley	90	<MRL
3	Yuma Valley	44	Not Detectable
4	Yuma Valley	<MRL	Not Detectable
5	Yuma Valley	62	<MRL
6	Yuma Valley	<MRL	Not Detectable
7	Yuma Valley	42	<MRL
8	Yuma Valley	58	Not Detectable
9	Yuma Valley	45	Not Detectable
10	Yuma Valley	78	<MRL
11	Gila Valley	52	<MRL
12	Gila Valley	48	<MRL
13	Gila Valley	63	<MRL
14	Gila Valley	63	<MRL
15	Gila Valley	39	Not Detectable
16	Gila Valley	77	<MRL
17	Gila Valley	52	<MRL
18	Wellton-Mohawk	55	<MRL
19	Wellton-Mohawk	43	<MRL
20	Wellton-Mohawk	64	<MRL
21	Wellton-Mohawk	55	<MRL
22	Wellton-Mohawk	56	<MRL
23	Wellton-Mohawk	56	<MRL
24	Wellton-Mohawk	65	<MRL

<MRL represents seemingly detectable peak but below level that can be quantitated.